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STUDIES ON THE PARASITISM OF FUSARIUM LINI BOLLEY[1]

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The development and distribution of resistant varieties of flax has aided materially in the solution of the flax wilt problem. However, resistant varieties are not always resistant. They may be resistant in one locality and not in another. This difference in resistance may be due partly to environmental conditions, as Tisdale (10) and Barker (4) have shown that wilt resistance is only relative and may be modified by environmental conditions. It has been demonstrated that the effect of environment may be overcome to some extent by early planting (4). However, the results given in a former abstract (6) and in this paper indicate that the differences in reaction of resistant varieties in different localities may be due also to physiologic specialization of the pathogene. If this is true, it will be necessary either to select resistant varieties for certain localities, or varieties resistant to all physiologic forms.

Since Tisdale (11), Barker (4), and Anderson (1) have reviewed the literature on flax wilt, it will be unnecessary to review it again in this paper.

Five varieties of flax, namely, N. D.[2] 40013 (C. I. 241); N. D. 3080 (C. I.

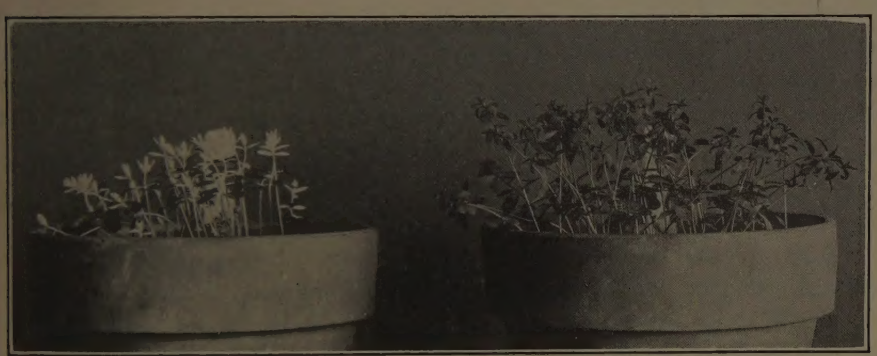

FIG. 1. Reaction to *Fusarium lini*, form 1, on Primost, Minn. 25 (C. I. 177), in pots. Check pot on the right.

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The writer desires to acknowledge his indebtedness to Dr. E. C. Stakman for his helpful advice and criticism.

[2] N. D. = North Dakota Agricultural Experiment Station accession number.
C. I. = Office of Cereal Investigations accession number.
Minn. = Minnesota Agricultural Experiment Station accession number.

275); Slope (C. I. 274); Winona, Minn. 182 (C. I. 179); and Chippewa, Minn. 181 (C. I. 178), when grown at Mandan, N. D., were highly resistant to flax wilt and yielded well, according to data of Stoa and Dillman (9). When grown at University Farm, St. Paul, Minn.,[3] on the other hand, the first three varieties were more or less susceptible to wilt and yielded less than the other two, which were highly resistant also at University Farm. Table 1 shows the striking differences in yields when the varieties were

TABLE 1.—*Yields in bushels per acre of flax varieties grown at Mandan, North Dakota, and University Farm, St. Paul, Minnesota*

Flax varieties	C.I.[a]	Yields in bushels per acre	
		Mandan, N. Dak.	Univ. Farm, St. Paul, Minn.
N. D. 40013	241	11.5	1.89
N. D. 3080	275	11.3	1.31
Slope	274	10.1	3.19
Winona, Minn. 182	179	10.4	9.01
Chippewa, Minn. 181	178	10.2	10.20

[a] C. I. = Office of Cereal Investigations accession number.

grown at University Farm, St. Paul, Minn., and the lack of significant differences at Mandan, N. D.

Inasmuch as there seemed to be strong circumstantial evidence of physiologic specialization in *Fusarium lini,* the writers attempted to ascertain whether the fungus actually was specialized, and to determine the number of forms, their morphologic characteristics, parasitic capabilities, degree of stability, and mode of action.

MATERIALS AND METHODS

Table 2 lists the sources of material for cultures of *F. lini.* Four varieties of flax (*Linum usitatissimum* L.), namely, Primost, Minn. 25 (C. I. 177); Winona, Minn. 182 (C. I. 179); N. D. 3080 (C. I. 275); and N. D. 40013 (C. I. 241) were used as differential hosts for inoculation experiments. The varieties were supplied by Dr. A. W. Henry, of the Division of Plant Pathology, University of Minnesota. Cultures, accession Nos. 1, 2, 4, to 17, inclusive, are of monosporous origin. These were obtained by picking up single germinating spores with a sterile platinum needle from poured agar plates. In the case of culture No. 3, a single chlamydospore-like body in a hyphal thread was picked up. Stock cultures were grown on potato dextrose agar and were kept at room temperature.

[3] Unpublished results furnished by Doctor A. W. Henry, University Farm, St. Paul, Minnesota.

TABLE 2.—*Source of materials for cultures of Fusarium lini isolated from flax*

Accession no.	Locality or origin	Transferred from	Collector or sender	Isolated by	Date isolated or transferred
1	Agronomy plot, St. Paul, Minn.		W. C. Broadfoot	W. C. Broadfoot	Aug. 2, 1924
2	Austin, Minn.		J. J. Christensen	W. C. Broadfoot	July 24, 1924
3	Cadillac, Sask.		R. G. Olgivie	W. C. Broadfoot	Nov. 6, 1924
4	Cadillac, Sask.		R. G. Olgivie	W. C. Broadfoot	Nov. 6, 1924
5	Crookston, Minn.		A. W. Henry	W. C. Broadfoot	Sept. 2, 1924
6	Crookston, Minn.		A. W. Henry	W. C. Broadfoot	Sept. 2, 1924
7	Fargo, N. D.	Minn. Herb. culture no. 53	H. D. Barker	H. D. Barker July 5, 1921	Aug. 30, 1924
8	Kenyon, Minn.	Minn. Herb. culture no. 52	H. D. Barker	H. D. Barker March 11, 1921	Aug. 30, 1924
9	Morris, Minn.		J. J. Christensen	W. C. Broadfoot	July 24, 1924
10	Plant Pathology plot, St. Paul, Minn.		W. C. Broadfoot	W. C. Broadfoot	Aug. 2, 1924
11	Red Lake Falls, Minn.		A. W. Henry	W. C. Broadfoot	Aug. 20, 1924
12	Saskatoon, Sask.		G. Boyd	W. C. Broadfoot	Nov. 6, 1924
13	St. Lawrence, Minn.		A. W. Henry	W. C. Broadfoot	Aug. 2, 1924
14	Winnipeg, Man.		G. R. Bisby	W. C. Broadfoot	Sept. 30, 1924
15	Winnipeg, Man.		G. R. Bisby	W. C. Broadfoot	Sept. 30, 1924
16	St. Paul, Minn.	Minn. Herb. culture no. 58	H. D. Barker	H. D. Barker May 20, 1921	Sept. 30, 1924
17	do	Minn. Herb. culture no. 60	H. D. Barker	H. D. Barker Nov. 25, 1921	Sept. 30, 1924
19	do	Minn. Herb. culture no. 95	O. S. Aamodt	O. S. Aamodt July, 1915	Mar. 6, 1925
20	do	Minn. Herb. culture no. 16	H. D. Barker	H. D. Barker 1919	Mar. 6, 1925

TABLE 3.—*Results of inoculating four varieties of flax with nine physiologic forms of Fusarium lini*

Physiologic form	Accession no.	Trial	Varieties and Reactions[a]													
			Primost, Minn. 25				Winona, Minn. 182				N. D. 3080				N. D. 40013			
			Surviving plants			Reaction	Surviving plants			Reaction	Surviving plants			Reaction	Surviving plants			Reaction
			Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials	
1	3	1	0	0.0		C	0	0.0		C	3	3.7		C	1	1.0		C
		2	0	0.0	0.0	C	0	0.0	0.0	C	0	0.0	1.9	C	0	0.0	0.5	C
2	7	1	2	2.0		S	7	10.9		S	8	10.0		S	9	9.8		S
		2	0	0.0	1.0	S	1	1.8	6.4	S	0	0.0	5.0	S	4	7.2	8.5	S
3	9	1	0	0.0		S	4	6.2		S	16	20.0		R	0	0.0		S
		2	2	3.8	1.9	S	9	17.9	11.5	S	16	27.5	23.5	R	10	12.4	6.2	S
4	8	1	6	5.7		S	23	36.6		R	5	6.3		S	17	18.8		S
		2	12	16.3	11.0	S	37	73.6	55.1	R	6	10.3	8.2	S	10	18.3	18.5	S
5	2	1	7	6.6		S	1	1.4		S	10	12.2		S	28	30.3		R
		2	4	7.1	6.8	S	2	4.2	2.8	S	2	3.9	7.9	S	19	35.0	32.6	R
6	6	1	11	10.5		S	15	23.8		R	3	3.7		S	38	41.8		R
		2	5	9.3	9.9	S	23	46.0	34.9	R	8	13.5	8.6	S	18	33.6	37.7	R
7	4	1	6	5.7		S	18	28.6		R	26	32.2		R	11	12.2		S
		2	9	16.4	11.0	S	26	52.1	40.4	R	22	37.8	35.0	R	5	9.4	10.8	S
8	5	1	25	23.7		R	39	61.9		R	11	13.5		S	11	13.5		S
		2	25	45.3	34.5	R	24	47.9	59.9	R	0	0.0	6.8	S	0	0.0	6.8	S
9	1	1	54	56.2		R	33	52.4		R	67	76.6		R	21	23.1		R
		2	19	34.4	45.3	R	12	23.9	38.1	R	19	32.6	54.6	R	30	55.5	39.3	R
Uninoculated check		1	106	100.0			61	100.0			81	100.0			91	100.0		
		2	55	100.0	100.0		50	100.0	100.0		58	100.0	100.0		54	100.0	100.0	

[a] R = Resistant; S = Susceptible; C = Susceptible and chlorotic above cotyledons.

The inoculum for soil inoculations was produced on a sterilized medium consisting of a mixture of sterilized wheat, oats, and barley seeds combined with an equal volume of water. An equal amount of inoculum of each culture was mixed with sterilized soil in twelve 4-inch pots. Seeds of differential hosts were surface disinfected by immersion for 10 minutes in a 1-1000 $HgCl_2$ solution, then washed in sterile distilled water and allowed to dry. In each pot in experiment 1, 50 seeds of one variety were sown, and 25 seeds per pot in experiment 2. The control pots were treated in the same way, except that sterilized uninoculated medium was mixed with the soil. Two inoculation experiments, each in triplicate, were made in the greenhouse, one in March and the other in June. The number of plants surviving in the control pots was considered as 100 per cent. Varieties were arbitrarily designated resistant if more than 20 per cent of the plants survived.

RESULTS

Various degrees of resistance and susceptibility appeared. On this basis nine physiologic forms of *F. lini* were recognized. The results of the inoculations with nine forms are summarized in table 3.

An examination of table 3 shows that forms 1 and 2 are somewhat similar in pathogenicity. However, form 1 causes the plants to lose their chlorophyll, a condition which is brought about by no other form (Fig. 1). When the plants are from 4 to 6 inches high the green color gradually disappears from all the tissues above the cotyledons until this portion of the plant eventually becomes entirely colorless. The line of demarcation is very sharp between the normal and "chlorophyll deficient" tissues (Fig. 2). Sections of these tissues show that the chloroplasts are decidedly deformed as well as colorless. The affected plant eventually dies. This condition was produced by artificial inoculation in the field as well as in the greenhouse. Form 1 also caused a similar condition on Manchuria barley, Victory oats, and Rosen rye. The green color gradually disappeared from all the tissues above the first leaf.

The forms are numbered in order of their virulence. All four varieties are susceptible to forms 1 and 2, which are the most virulent. Only one variety, a different one for each of the forms, is resistant to forms 3, 4, and 5. Similarly, two varieties are resistant to forms 6, 7, and 8. Form 9 is the least virulent. All four varieties are resistant to this form, although it kills some of the plants of all varieties. For convenience in distinguishing physiologic forms of *F. lini*, an analytical key of the simple dichotomous type is presented in table 4.

Table 5 gives the place of collection of the nine forms of *F. lini* used in the experiments. No attempt was made to determine the prevalence and

TABLE 4.—*Analytical key to physiologic forms of Fusarium lini*

Chlorosis absent		
Primost, Minn. 25 (C. I. 177)	Resistant	
N. D. 3080 (C. I. 275)	Resistant	Form 9
N. D. 3080	Susceptible	Form 8
Primost	Susceptible	
N. D. 3080	Resistant	
Winona, Minn. 182 (C. I. 179)	Resistant	Form 7
Winona	Susceptible	Form 3
N. D. 3080	Susceptible	
N. D. 40013 (C. I. 241)	Resistant	
Winona	Resistant	Form 6
Winona	Susceptible	Form 5
N. D. 40013	Susceptible	
Winona	Resistant	Form 4
Winona	Susceptible	Form 2
Chlorosis present		Form 1

distribution of the different forms. However, certain of the forms studied appeared to be more widely distributed than others. Thus forms 2, 3, and 7 were found both in the United States and Canada, while form 1 was found only in Saskatchewan and the other forms in Minnesota alone. But it must be borne in mind that only very few collections were made, and further studies might show the situation to be entirely different.

TABLE 5.—*Place of collection of nine physiologic forms of Fusarium lini*

Accession no.	Physiologic form	Place collected
3	1	Cadillac, Saskatchewan
7	2	Fargo, North Dakota
12		Saskatoon, Saskatchewan
13		St. Lawrence, Minnesota
20		University Farm, St. Paul, Minnesota
9	3	Morris, Minnesota
14		Winnipeg, Manitoba
15		Winnipeg, Manitoba
8	4	Kenyon, Minnesota
2	5	Austin, Minnesota
19		University Farm, St Paul, Minnesota
6	6	Crookston, Minnesota
11		Red Lake Falls, Minnesota
4	7	Cadillac, Saskatchewan
17		University Farm, St. Paul, Minnesota
5	8	Crookston, Minnesota
1	9	Agronomy plot, University Farm, St. Paul, Minnesota
10		Plant Pathology plot, University Farm, St. Paul, Minnesota

MORPHOLOGY OF THE PHYSIOLOGIC FORMS OF FUSARIUM LINI

The results of these experiments indicate clearly that there are physiologic forms of *F. lini*. Experiments were then made to determine whether there are differences in morphology as well as in pathogenicity. The nine forms of *F. lini* were grown on potato plugs, steamed rice, hard oat agar, sweet clover stems, alfalfa stems, 2 per cent potato dextrose agar, and 5 per cent potato dextrose agar, all in test tubes, according to the formulae recommended by Wollenweber, Sherbakoff, Reinking, Johann, and Bailey (12) for the identification of species of *Fusarium*. *F. lini* was first described by Bolley (5) from cultures grown on slightly acid peptone agar. Such an agar was made, consisting of 2 grams of peptone, 2 grams of sugar, 2 grams of agar, a few drops of lactic acid, and 1,000 cc. of water.

Spore measurements were made with an eye-piece micrometer. A 10 per cent aqueous solution of glycerine was used for mounting the spores on the glass slides.

Comparisons of mean length and width of different magnitude of spore populations of Fusarium lini, form 1, grown in the same medium

Separate spore populations of 50, 100, and 200 spores of *F. lini*, form 1, grown on 5 per cent potato dextrose agar 56 days old, were measured for length and width. From the data thus obtained, biometric constants were calculated by the assumed mean method (3). They are given in table 6. The differences in mean length and width of each spore population were then compared in relation to their probable errors (table 7). The differences between lengths of 50 spore and those of 100 and 200 spore populations are more than three times the probable error of the difference. When the mean length of 100 spores is compared with that of 200, the difference in the two means is less than one times the probable error of the difference. It would seem, therefore, that 50 spores may not constitute a representative random sample for drawing conclusions, but that 100 spores will probably be sufficient for a comparison of the spore lengths of *F. lini*. Similarly, it appears that measurements of 50 spores might be sufficient to determine spore width. However, in all cases of further comparative spore measurements, 100 spores were individually measured for length and width.

Comparisons of the length and width of 100 spores of Fusarium lini, form 1, grown on different media

Measurements were made of 100 spores of form 1 grown on 3 different media and are listed in table 8. In one case the spores were taken from a culture of form 1, 14 days old, grown on slightly acid peptone agar. The cultures grown on sweet clover stems and on 5 per cent dextrose agar were 56 days old. The mean length of the spores from the 5 per cent potato dex-

TABLE 6.—*Variations and constants for length and width of spores of Fusarium lini, form 1, based on measuring populations of different magnitudes, under similar conditions*

Medium	Age of culture in days	Magnitude of population samples	Spore classes according to length in microns						Constants			Spore classes according to width in microns						Constants		
			20	25	30	35	40	45	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error	3.0	3.3	3.6	3.9	4.2	4.5	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
5 pct. potato dextrose agar	56	50	8	16	11	10	4	1	28.90 ± 0.61	6.35 ± 0.43	21.97 ± 1.38	4	15	13	10	5	3	3.64 ± 0.04	0.40 ± 0.03	10.98 ± 0.74
do	56	100	33	34	16	10	4	3	26.35 ± 0.44	6.57 ± 0.31	24.93 ± 1.19	4	39	28	21	5	3	3.58 ± 0.02	0.33 ± 0.02	9.22 ± 0.44
do	56	200	53	78	43	16	9	1	26.38 ± 0.27	5.65 ± 0.19	21.40 ± 0.77	9	78	74	27	8	4	3.54 ± 0.01	0.30 ± 0.01	8.47 ± 0.28

TABLE 7.—*Summary of comparisons between means and coefficients of variability for length and width of 100 spores of Fusarium lini, form 1, based on data in table 6*

Conditions compared: magnitude of population sample	Length				Width			
	Means		Coefficient of variability		Means		Coefficient of variability	
	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference
50 and 100	2.55 ± 0.75	3.40	2.96 ± 1.90	1.50	0.06 ± 0.04	0.13	1.76 ± 0.86	2.04
50 and 200	2.52 ± 0.66	3.80	0.56 ± 1.65	0.33	0.10 ± 0.04	2.50	2.51 ± 0.71	3.16
100 and 200	0.03 ± 0.52	0.05	0.47 ± 1.39	0.33	0.04 ± 0.03	1.53	0.75 ± 0.52	1.43

TABLE 8.—*Variations and constants for length and width of 100 spores of Fusarium lini, form 1, on different media*

Medium	Age of culture in days	Spore classes according to length in microns							Constants			Spore classes according to width in microns						Constants		
		15	20	25	30	35	40	45	Mean and probable error	Standard deviation and prob-	Coefficient of variability and probable error	3.0	3.3	3.6	3.9	4.2	4.5	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
5 pet. potato dextrose agar	56		33	34	16	10	4	3	26.35 ± 0.44	6.57 ± 0.31	24.93 ± 1.19	4	39	28	21	5	3	3.58 ± 0.02	0.33 ± 0.02	9.22 ± 0.44
Sweet clover	56	24	44	20	10	1	1		21.15 ± 0.34	5.09 ± 0.24	24.06 ± 1.15	10	51	29	7	3		3.43 ± 0.02	0.26 ± 0.01	7.63 ± 0.36
Peptone agar	14		8	28	33	22	7	2	29.10 ± 0.38	5.66 ± 0.27	19.50 ± 0.93	8	34	29	21	8		3.56 ± 0.02	0.33 ± 0.02	9.30 ± 0.44

TABLE 9.—*Summary of comparisons between means and coefficients of variability for length and width of 100 spores of Fusarium lini, form 1, based on data in table 8*

Conditions compared: media	Length				Width			
	Means		Coefficient of variability		Means		Coefficient of variability	
	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference
5 pct. potato dextrose agar and sweet clover	5.20 ± 0.55	9.29	0.87 ± 1.65	0.52	0.15 ± 0.03	5.35	1.59 ± 0.57	2.77
5 pct. potato dextrose agar and peptone agar	2.75 ± 0.58	4.70	5.43 ± 1.51	3.59	0.02 ± 0.02	0.83	0.08 ± 0.68	0.12
Sweet clover and peptone agar	7.95 ± 0.51	15.49	4.56 ± 1.48	3.09	0.13 ± 0.03	4.64	1.67 ± 0.57	2.91

trose culture was found to be 26.35 ± 0.44 microns; for the spores from sweet clover culture the mean length was 21.15 ± 0.34 microns. The difference between these means is more than nine times the probable error of the difference, which apparently is quite significant. The difference in the mean width of spore cultures grown on 5 per cent potato dextrose agar and on the sweet clover was found to be more than five times the probable error of the difference. This again apparently is significant. The difference in the mean length and width of spores from cultures grown on sweet clover

FIG. 2. Reaction to *Fusarium lini*, form 1, on individual plants of Primost, Minn. 25 (C. I. 177), taken from inoculated soil. Plants on the right are from the check pots.

and from those grown on slightly acid peptone agar were found to be respectively more than fifteen and four times the probable error of the difference. These differences also are apparently very significant, certainly with respect to the mean length. The difference in mean length and width of spores from cultures grown on 5 per cent potato dextrose agar and slightly acid peptone agar are respectively more than four times and less than one times the probable error of the difference. The difference in mean length is apparently significant; whereas the difference in width probably is not. These results are summarized in table 9. Larger or smaller differences might have been obtained if spores had been grown on other media and similarly compared. Such differences as these undoubtedly account for conflicting data on spore size given by different authors for the same organism. It is evident that there may be a marked and significant difference in spore size of a given form of *F. lini* when grown on several different media. Therefore it is important to maintain uniform cultural conditions with respect to media and environment when the comparative morphology of forms of *F. lini* is studied.

Comparisons of the mean length and width of 100 spores of nine forms of Fusarium lini from cultures grown in the same medium

Table 10 gives a summary of the measurements of nine forms from cultures 7 to 14 days old grown on slightly acid peptone agar. Form 5 was measured with a screw micrometer. The mean length varied from 20.10 ± 0.33 microns for form 7, to 35.95 ± 0.42 microns for form 5. The mean width varied from 3.27 ± 0.03 microns for form 2, to 4.08 ± 0.04 microns for form 5. These differences are apparently significant. However, forms 2 and 7 have mean lengths of 20.70 ± 0.27 microns and 20.10 ± 0.33 microns respectively; in this case the differences are smaller. Greater differences might have occurred if thousands of spores had been measured. Therefore spore size alone is not a sufficient basis for a definite separation of forms of *F. lini.*

It has been shown that the same form of *F. lini* differs in spore size when grown on different media. On the same medium, some of the forms differ significantly, while others do not. Therefore spore size of forms of *F. lini* is not an accurate criterion for separating them into distinct morphological categories; but it indicates that there are inherent differences in the spore dimensions of different physiologic forms when cultured on the same medium under identical environmental conditions.

Comparison of cultural and structural characteristics of nine forms of Fusarium lini grown on seven different media

Tables 11 to 17 inclusive summarize the structural and cultural characteristics of nine forms of *F. lini* cultured on seven media in test tubes, as

TABLE 10.—*Variations and constants for length and width of 100 spores of each of the nine physiologic forms of Fusarium lini grown on slightly acid peptone agar*

Physiologic form	Accession no.	Spore classes according to length in microns								Constants			Spore classes according to width in microns									Constants		
		15	20	25	30	35	40	45	50	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error	2.7	3.0	3.3	3.6	3.9	4.2	4.5	4.8	5.1	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
1	3		8	28	33	22	7	2		29.10 ± 0.38	5.66 ± 0.27	19.50 ± 0.93		8	34	29	21	8				3.56 ± 0.02	0.33 ± 0.02	9.30 ± 0.44
2	7	19	54	22	4	1				20.70 ± 0.27	4.00 ± 0.19	19.30 ± 0.92		6	26	32	28	8				3.62 ± 0.02	0.31 ± 0.02	8.56 ± 0.41
3	9	2	17	57	22	2				25.25 ± 0.25	3.70 ± 0.18	14.65 ± 0.70		20	16	41	10	6	5	2		3.27 ± 0.03	0.43 ± 0.02	13.14 ± 0.63
4	8		13	24	29	32	2			29.30 ± 0.40	5.33 ± 0.25	18.19 ± 0.87		2	18	36	27	16	1			3.42 ± 0.02	0.46 ± 0.02	13.45 ± 0.64
5	2		2	7	22	24	30	14	1	35.95 ± 0.42	6.29 ± 0.30	17.50 ± 0.83	2	4	11	8	27	9	27	8	4	4.08 ± 0.04	0.61 ± 0.03	15.00 ± 0.82
6	6	4	44	48	2	1	1			23.75 ± 0.25	3.70 ± 0.18	15.60 ± 0.74			16	46	33	4	1			3.68 ± 0.02	0.24 ± 0.01	6.52 ± 0.31
7	4	37	31	28	2	1	1			20.10 ± 0.34	4.95 ± 0.24	24.60 ± 1.17		4	47	37	11	1				3.47 ± 0.02	0.23 ± 0.01	6.63 ± 0.32
8	5		1	37	50	11	1			29.70 ± 0.44	3.65 ± 0.31	12.30 ± 0.59			20	44	31	4	1			3.67 ± 0.02	0.25 ± 0.01	6.81 ± 0.33
9	1		22	39	23	12	2	2		26.95 ± 0.38	5.65 ± 0.27	21.00 ± 1.00		3	28	31	32	4	1	1		3.64 ± 0.01	0.10 ± 0.01	2.75 ± 0.13

recommended by Wollenweber *et al* (12). The percentages of spores falling in the septation classes, as listed, were obtained by examination of 100 spores as practiced by Appel and Wollenweber (2) and Sherbakoff (8). The spore sizes are given on the basis of an average of 10 macroconidia, individually measured for length and width. Spore dimensions were not given for less than 10 spores. The presence or absence of microconidia, macroconidia, terminal and intercalary chlamydospores, the color of mycelium, and substrate was quite variable. Sporulation was not common for all the forms on any one of these media. Forms 1 and 6 sporulated on only two of the media; form 4 on three media; forms 3, 5, 7, and 8 on four media; form 2 on five of the media; and form 9 on seven of the media. There was also great variation in other microscopical characters.

TAXONOMIC POSITION OF FUSARIUM LINI IN FORM GENUS FUSARIUM

Some of the characteristics common to section Elegans (12) of form genus *Fusarium* and *F. lini* are thin-walled microconidia, cylindrical to long ellipsoid, not pyriform, and not catenulate on aerial mycelium. The microconidia are usually present and dominately 0-septate. Macroconidia are usually pedicillate and attenuate at the top end. Terminal and intercalary chlamydospores are usually present. There is no blue or green color in the conidia even as a diffusion from the stroma. The stroma is principally vinaceous to lilac on artificial media.

PHYSIOLOGY OF PHYSIOLOGIC FORMS OF FUSARIUM LINI

Effect of Temperature on Rate of Growth

In order to determine whether the physiologic forms of *F. lini* used in this work could be recognized on the basis of reaction to temperature, a series of tests was made as follows:

Petri dishes of uniform size were poured uniformly with slightly acid peptone agar. At the end of two days, contaminated plates were discarded, and to the others was transferred an approximately uniform amount of mycelium from stock cultures of forms 2, 3, 4, and 5. The plates were incubated at room temperatures for two days before distribution to the various temperature incubators. At the end of 10 days, the diameter of each colony was measured and averaged. The experiment was carried out in duplicate.

The results are illustrated in figure 3. The difference in growth made by the four forms at the various temperatures evidently falls within the limits of experimental error. That is, none of the forms tested could be distinguished from each other on the basis of growth at various temperatures.

TABLE 11.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction[d] of nine physiologic forms of Fusarium lini grown on potato plugs under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Microconidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa							
		0	1	2	3		Terminal	Intercalary	
1	3	Macroconidia, if any, rare				−	+	+	30
2	7	5 −	72 20.86 x 3.33	5 −	18 30.64 x 3.33	+	−	−	34
3	9	2 −	7 −	3 −	85 38.2 x 3.52	+	+	+	51
4	8	Macroconidia, if any, rare				−	−	−	51
5	2	9 −	9 −	9 −	73 39.28 x 3.30	+	+	+	30
6	6		Rare			+	+ Rare	+	50
7	4	Macroconidia, if any, rare				−	+	+	43
8	5	80 16.32 x 3.29	19 17.78 x 3.28			+	+	+	48
9	1	93 8.39 x 1.17	7 −						

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the spore size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

TABLE 12.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on hard oat agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b] — Number of septa					Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		0	1	2	3	4		Terminal	Intercalary		Mycelium	Substrate
1	3	Macroconidia, if any, rare					–	+	+	30	White to light pink	Very light pink
2	7	94 16.32 x 3.26	6 –				+	+	+	43	White	Unchanged
3	9	3 –	7 –	6 –	51 28.86 x 3.39	33 44.66 x 3.36	+	+	+	51	do	do
4	8	19 17.28 x 3.36	35 22.49 x 3.36	18 25.43 x 3.82	28 34.43 x 3.82		+	+	+	51	do	do
5	2	60 28.3 x 3.33	8 –	2 –	30 30.64 x 3.29		+	+	+	30	do	do
6	6		Rare				+	+	+	50	do	do
7	4	23 18.91 x 3.39	35 20.54 x 3.29	6 –	36 30.32 x 3.29		+	+	+	43	do	do
8	5	34 16.63 x 3.29	30 16.63 x 3.52	5 –	31 27.71 x 3.72		+	+	+	48	do	do
9	1	65 7.38 x 1.22	31 6.75 x 1.16	4 –			+	+	+	29	do	do

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the spore size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

TABLE 13.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on 2 per cent potato dextrose agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa										
		0	1	2	3	4		Terminal	Intercalary		Mycelium	Substrate
1	3	Macroconidia, if any, rare					−	+	+	31	White, light pink	Light pink
2	7	25 17.28 x 3.39	62 20.54 x 3.39	11 29.01 x 3.36			+	+	+	43	White, appressed	Unchanged
3	9	4 −	10 21.84 x 3.46	4 −	75 38.92 x 3.26	7 −	+ Rare	+	+	51	White, appressed	do
4	8	Rare	Rare	Rare			+ Very abundant	+	+	51	White	do
5	2		Rare	Rare	Rare		+	+	+	30	White, appressed	do
6	6	17 17.28 x 3.67	55 17.28 x 3.39	6 −	22 31.30 x 3.33		+	+	+	50	White	do
7	4		60 19.49 x 3.29	7 −	33 30.32 x 3.42	Rare	+	+ Very abundant	+	43	do	do
8	5	1 −	4 −	2 −	93 38.26 x 3.59		+	+	+	48	do	do
9	1	51 6.26 x 1.17	41 7.33 x 1.17	7 −	1 −		+	+	+	30	do	do

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

TABLE 14.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on 5 per cent potato dextrose agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa						Terminal	Intercalary		Mycelium	Substrate
		0	1	2	3	4						
1	3	3 –	5 –	9 –	83 31.3 x 4.01		+	+	+	30	White	Dark brown
2	7	Macroconidia, if any, rare					–	+	+	43	White, appressed	Unchanged
3	9	4 –	42 22.17 x 3.52	20 26.08 x 3.72	31 37.29 x 3.62	3 –	+	+	+	51	do	do
4	8	Rare	Rare				+ Very abundant	+	+	51	do	do
5	2	Macroconidia, if any, rare					+	–	–	30	do	do
6	6	Rare					+	+ Rare	+ Rare	50	White	do
7	4	Macroconidia, if any, rare					+	–	–	43	do	do
8	5	23 24.2 x 3.32	45 24.78 x 3.49	3 –	27 28.04 x 3.52		+	+ Rare	+ Rare	50	do	Very dark brown
9	1	99 7.38 x 1.22	1 –				+	–	–	30	do	Unchanged

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.

– indicates absence of microconidia or chlamydospores.

TABLE 15.—*The sporulation, variation in number of septa of macroconidia, the size of macronidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on steamed rice under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa					Terminal	Intercalary		Mycelium	Substrate
		0	1	2	3						
1	3	Macroconidia, if any, rare				−	+	+	30	White	Light yellow
2	7	3 −	78 24.12 x 3.36	5 −	14 30.97 x 3.42	+	+ Rare	+ Rare	34	do	Light pink
3	9		Rare	Rare		+	+	+	51	White, appressed	Light brown
4	8	Rare	Rare			+	+ Rare	+ Rare	51	do	do
5	2	Macroconidia, if any, rare				+	+	+	30	White	do
6	6	Macroconidia, if any, rare				+	−	−	50	do	White with trace of purple at line of diffusion
7	4	Macroconidia, if any, rare				+	+	+	43	Very light brown	Unchanged
8	5	Macroconidia, if any, rare				+	−	−	45	White	White with trace of purple at line of diffusion
9	1	81 5.59 x 1.21	19 5.91 x 1.23			+	−	−	29	do	Light pink

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

TABLE 16.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color[d] reaction of nine physiologic forms of Fusarium lini grown on steamed sweet clover stems under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa								
		0	1	2	3	4		Terminal	Intercalary	
1	3	1 –	8 –	25 23.15 x 3.49	66 22.71 x 3.62		+	+	+	30
2	7	6 –	6 –	4 –	88 32.63 x 3.39		+	+	+	34
3	9	Rare	Rare				+	+	+	51
4	8	Macroconidia, if any, rare					–	+ Very abundant	+	51
5	2	45 20.21 x 3.39	46 18.26 x 3.52	4 –	5		+	+	+	30
6	6	4 –	64 18.62 x 3.39	4 –	28 27.78 x 3.52		+	+	+	50
7	4	3 –	15 19.23 x 3.39	10 23.15 x 3.69	72 29.01 x 3.91	Rare	+	+ Very abundant	+	43
8	5	Culture dried up								
9	1	12 8.56 x 1.16	68 7.18 x 1.18	17 8.83 x 1.21	3 –		+	+	+	29

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

TABLE 17.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction[d] of nine physiologic forms of Fusarium lini grown on steamed alfalfa stems under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Microconidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa							
		0	1	2	3		Terminal	Intercalary	
1	3	Macroconidia, if any, rare				−	+ Very abundant	+	30
2	7	Macroconidia, if any, rare				−	+	+	31
3	9	Rare	Rare	Rare		+	+ Rare	+ Rare	51
4	8	50[e] 17.93 x 3.33	22 21.84 x 3.29	2 −	26 28.09 x 3.33	+	−	−	50
5	2	54 26.21 x 3.39	31 29.9 x 3.39	9 −	6 −	+	+	+	30
6	6	Macroconidia, if any, rare				+	+	+	50
7	4	15 22.85 x 3.72	34 20.86 x 3.39	22 23.47 x 3.69	29 27.39 x 3.65	+	+	+	43
8	5	Rare	Rare			+	+	+	50
9	1	56 6.97 x 1.15	41 7.29 x 1.16	2 −	1 −	+	+	+	29

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

[e] Based on 50 spores.

TABLE 18.—*Comparative cultural characteristics of nine physiologic forms of Fusarium lini when grown on the same and different media under the same conditions*

Physiologic form	Accession no.	Medium	Mycelium								Color		Sporulation				Age of colonies in days				
			Plumose	Appressed	Cottony	Floccose	Woolly	Tufted	Silky	Cobwebby	Mycelium	Substrate	Abundant	Medium	Sparse	Absent	4	6	8	10	12
																	Size of colonies in mm.				
1	3						+				White to pink	Light brown				+	9	15	22	29	36
2	7	Potato					+				do	do	+				14	19	25	31	37
3	9				+	+	+				do	do	+				16	21	27	34	31
4	8	glucose			+		+				do	do	+				15	20	26	33	40
5	2						+				do	do	+				16	21	26	33	39
6	6	agar	+		+						do	do	+				17	23	30	37	44
7	4				+						do	Brown	+				16	22	29	36	43
8	5					+	+			+	do	Light brown	+				17	24	31	38	45
9	1				+						White	do	+				13	16	21	26	31
1	3							+			White	Brown			+		5	7	8	9	10
2	7	Prune		+					+		do	Unchanged		+			12	17	22	29	36
3	9			+					+		do	do		+			16	22	29	36	43
4	8			+					+		do	do			+		14	19	25	32	39
5	2			+					+		do	do			+		14	19	25	32	34
6	6	agar		+					+		do	do	+				14	19	26	33	40
7	4			+					+		do	Brown		+			17	23	29	36	43
8	5			+					+		do	Unchanged		+			15	20	26	32	38
9	1			+					+		do	do			+		11	14	19	24	30
1	3			+					+		White	Unchanged			+		10	17	23	29	35
2	7			+					+		do	do		+			14	19	25	31	37
3	9	Corn		+					+		do	do		+			16	22	25	36	44
4	8			+					+		do	do		+			15	20	26	33	40
5	2	meal		+					+		do	do			+		14	19	25	32	39
6	6			+					+		do	do	+				15	20	26	32	38
7	4	agar		+					+		do	do	+				14	20	26	33	40
8	5			+					+		do	do	+				15	20	26	32	38
9	1			+					+		do	do			+		12	17	22	27	33

TABLE 19.—*Comparative cultural characteristics of nine physiologic forms of Fusarium lini when grown on slightly acid peptone agar under the same conditions*

Physiologic form	Acc. no.	Color		Zoning			Mycelium				Diameter of colonies in mm. at end of eight days
		Mycelium	Substrate	Distinct	Moderate	Faint	Woolly	Cottony	Tufted	Appressed at margin	
1	3	Pink	Reddish		+			+			42
2	7	do	Unchanged			+	+			+	65
3	9	do	Light yellow			+	+			+	65
4	8	do	Unchanged			+	+			+	62
5	2	Light yellow	Light yellow			+	+			+	65
6	6	Pink	do	+			+		+		73
7	4	do	do			+		+			70
8	5	do	do	+			+		+		72
9	1	White	Cream	+			+		+		50

Effect of Medium on Cultural Characteristics

Appel and Wollenweber (2), Sherbakoff (8), and others have used the cultural characteristics of micro-organisms on different media to distinguish species of *Fusarium* which could not be differentiated on a morphological basis.

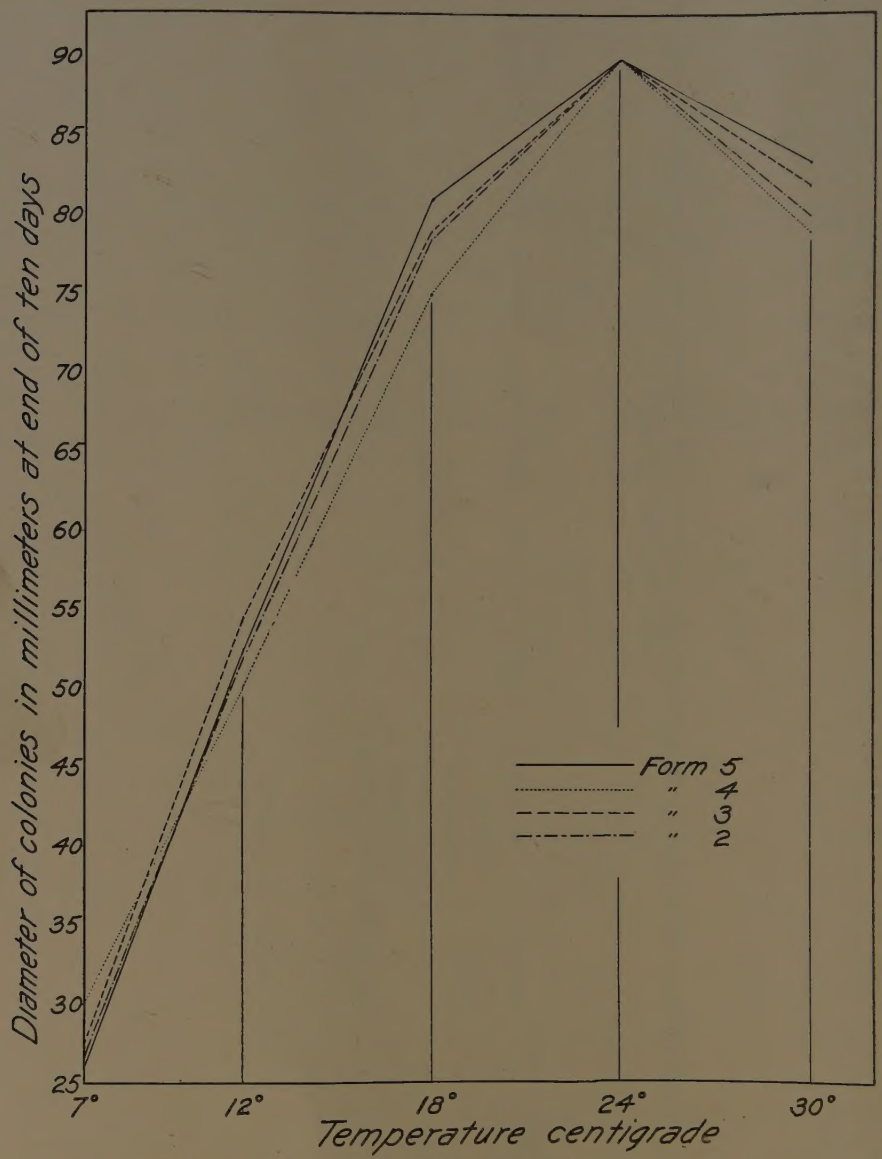

FIG. 3. Growth of four forms of *Fusarium lini* at the end of ten days on slightly acid peptone agar at various temperatures.

In order to compare their cultural characteristics, the nine forms of *F. lini* were grown on three different kinds of media: potato glucose agar, consisting of 20 grams of freshly peeled potato, 5 grams of sugar, 2 grams of agar, and 100 cc. of water; and synthetic cornmeal agar, and prune agar both manufactured by the Digestive Ferments Company, Detroit, Michigan. Triplicate plates of each medium were inoculated with small and, as nearly as possible, equal portions of mycelium of each form. Each medium was made in one lot, tubed, sterilized, and poured at the same time. All of the plates were kept under the same general environmental conditions. The nine forms listed in table 18 can be differentiated macroscopically, although with difficulty, by the following characters: color of mycelium and substrate, mycelial characters, sporulation, and rate of radial growth.

The organism which Bolley (5) described as a new species of *F. lini* was cultured on a slightly acid peptone agar. For this reason the cultural characteristics of nine forms of *F. lini* grown on slightly acid peptone agar are given in table 19.

Spore Germination

The process of spore germination in *F. lini* has been described by Bolley (5). The writer observed that one or more germ tubes, seldom more than two, are sent out from any portion of the spore, although usually from the ends. The size and number of germ tubes vary according to media and temperature.

TABLE 20.—*Summary of the percentage of germination of conidia of Fusarium lini, form 5, at various temperatures, in distilled water, with and without the addition of flax tissue, at the end of twelve hours*

Temperature in degrees C.	Flax tissue[a]	Percentage of germination				
		Trial				Average
		1	2	3	4	
7	–	16	12	10	12	12.50
	+	42	24	40	40	39.00
12	–	81	85	84	85	83.75
	+	83	90	86	90	87.25
18	–	86	84	84	82	84.00
	+	88	86	88	90	83.00
25	–	76	78	76	76	76.50
	+	82	90	86	90	87.00
30	–	71	73	74	90	90.00
	+	97	96	98	98	97.25
35	–	0	0	0	0	0
	+	0	0	0	0	0

[a] – indicates no flax tissue added; + indicates tissue added.

12. The 9 forms can be distinguished with difficulty by macroscopic examination when grown on 3 different media under the same conditions.

13. The minimum time required for spore germination in distilled water at 20–21° C. was found to be 2 hours and 5 minutes for culture No. 14. Germination occurred in 1 hour and 45 minutes when flax tissue was added to the medium. Best germination in distilled water occurred between 12° C. and 30° C.; in tap water at 30° C. Flax tissue when added to the medium increased germination in all cases. No germination occurred above 35° C. Germ tubes of spores at 12° C. were shorter than those at higher temperatures. Some germination took place at 7° C. The percentage of germination was higher in distilled water than in tap water.

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